Resumen por el autor, Benjamin T. Nelson.

El número de glomérulos en el riñón del conejo adulto.

El autor ha contado los glomérulos en riñones, en los cuales dichos elementos se habían teñído previamente mediante coloración supravital con verde janus. En uno de ellos ha contado el autor el número total, que resultó ser 163,075. En otros ocho riñones procedentes de cuatro animales, cálculos basados en el peso total de la corteza y en el recuento de un riñón previamente pesado han resultado en cifras que oscilan entre 118,160 y 168,-966. Con una sola excepción el número de glomérulos encontrado excede 154,000, y el número medio para todos ellos, incluyendo el riñón contado in toto es 157,180. El número de glomérulos en cada riñón del conejo adulto es por consiguiente unos 160,000, próximamente.

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THE NUMBER OF GLOMERULI IN THE KIDNEY OF THE ADULT RABBIT

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The number of glomeruli in a kidney is a measure of the number of uriniferous tubules, and as such a necessary factor in estimating the relative surface of glomerular and tubular epithelium concerned in the secretion of the urine. It is also an important element in Brodie's computation of the pressure necessary at the glomerular end of the tubule to drive the urine along the tubule at the rate at which it is secreted in diuresis.

The earliest enumeration of the tubules is credited to Eysenhardt (1818), whose article I have, unfortunately, been unable to consult. According to Huschke ('44), Eysenhardt estimated the tubules of the human kidney at 42,000,000—a number which Huschke criticised as probably much too high because of the enumeration of blood vessels as tubules. Through an error Huschke is usually credited by authors as the first to enumerate the glomeruli, but his article in Oken's Isis, vol. 21, 1828, to which reference is made by Brodie, Policard, and others, contains no mention of the number, except the remark that they are more numerous, relative to mass, in the young than in the adult. Not until 1844, however, did Huschke discuss the actual number of tubules in the human kidney. In his article entitled "Eingeweidelehre" in Sömmering's Bau des menschlichen Körpers he said: "Every kidney lobe contains about 700 kidney lobules and each lobule about 200 cortical canals. If the kidney has 15 lobes that would give it 10,500 lobules and 2,100,000 cortical canals."

In his classical work on the kidney published in 1865, Schweigger-Seidel ('65) describes his method of determining the number of glomeruli in the kidney of the pig and discusses the results.

After carefully separating the cortical substance from the rest of the renal substance, he found, in a kidney weighing 120.5 grams, 102 grams of cortex. Small weighed portions of this cortex were teased and macerated in hydrochloric acid to permit the separation of the glomeruli for counting. In a total weight of 15.5 cgm. he found 720 glomeruli, and calculated the total content of the kidney as 473,200 glomeruli.

Peter's ('09) estimate of the number of tubules in the cat is based on the mode of branching of the ducts. He says: "Bei der Katze ergeben sich stets 4 initiale Aeste und meist 7 zentrale Teilungen; auf einen ins Becken mündenden Gang entfallen damit (2^8). 4 Kanälchen, d.h. 1024; nehmen wir die Zahl der Sammelröhren 1. Ordnung wie beim Hund auf 200–300 an, so ergäbe dies für jede Niere 200,000–300,000 Harnkanälchen." The number of tubules arising from a duct of the first order, 1024, was incorrectly quoted by Policard ('08) as Peter's estimate for the whole kidney.

Miller and Carlton in 1895 made an enumeration of the glomeruli in the cat kidney, based on a previous determination of the average volume of the cortex. They cut sections in series at a thickness of 0.10 mm. of a kidney injected with Prussian-blue gelatin. The outline of the section traced on paper and measured with a planimeter gave the area. They found that 50 per cent to 83 per cent of the glomeruli represented the actual number, the rest representing duplicate counts of glomeruli appearing in more than one section. They computed the content of an average volume kidney of 12.9 cc. containing 9.03 cc. of cortex in one estimation as from 9,183.49 to 15,325.13 glomeruli. Another estimation gave them from 13,288 to 22,220 glomeruli. The mean of these estimates is 15,664.

Brodie ('14) in 1914, with the assistance of Miss M. G. Thackrah, estimated the glomerular content in two dog kidneys, using a method similar to Miller and Carlton's. Brodie and Thackrah, however, cut a complete series of sections, 8 μ thick, of previously weighed pieces of cortex. In these sections the total number of glomeruli was counted and divided by the number of sections in which on the average a single glomerulus would

appear. In this way a ratio of number of glomeruli to weight was obtained from which the whole content of the cortex could easily be computed. The first dog weighed 11 kgrm., its right kidney weighed 34.5 grams, and the total number of glomeruli was 142,000. A kidney of a second dog, weighing a little over 8 kgrm., contained 125,000 glomeruli.

It is difficult to find a basis for comparison of these results because in the case of the cat only have we estimates made by two investigators. In this case the estimate of Peters is more than tenfold that of Miller and Carlton. The method employed by Miller and Carlton, however, involved the actual count of the glomeruli in series, and thus is less open to objection than that of Peters, who used in his computation a factor obtained from study of the dog's kidney.

If we compare in so far as is practicable the results of Miller and Carlton on the cat with those of Brodie on the dog, we find that Brodie's result, though agreeing fairly well with the estimate of Schweigger-Seidel on the pig, involves a content per milligram of kidney approximately three times as great as that of Miller and Carlton. The recent work of Bremer ('16) on the activity of the mesonephros in the embryos of various mammals, as indicated by the number and size of the glomeruli and by the increase in number and size of them in progressive phases of embryonic development, may throw some light on the variations in glomerular content of the kidneys of different species of mammals. Bremer found "that the different embryos can be classed as those which retain a functional wolffian body until the kidney is ready to take up the work of excretion, and those in which the wolffian body disappears early, before the kidney has developed active glomeruli." He finds also in the placenta of the latter group evidences of anatomical specialization for interchange with the maternal circulation, which justify the assumption of an excretory function on the part of the placenta. Thus it appears probable that the development of the kidney may be influenced by the concurrent existence, functional capacity, and history of other excretory mechanisms. Bremer also points out that within each of the classes which he recog-

nizes, "individual animals are provided with a very varying amount of excreting surface, showing presumably varying types of metabolism." The discussion of these interesting correlations must, however, be postponed, pending a confirmation by newer and better methods of the counts of glomeruli made in other mammals, which must in addition be supplemented by a careful quantitative study of the relative total glomerular surfaces—a study which involves a consideration not only of the number, but also of the size and lobulation of glomeruli.

My determinations have been made by a method essentially similar to that employed by Schweigger-Seidel, except that in most cases I counted the glomeruli in a much larger percentage of the cortex and, in two cases counted the glomeruli in the entire cortex of an adult rabbit's kidney. In addition, the glomeruli were stained so that there was no difficulty in seeing them and distinguishing them from tubules, which might be difficult in the unstained kidney used by Schweigger-Seidel.

The method is essentially the same as that employed by Bensley in the enumeration of the islands of Langerhans in the pancreas. Janus green B when injected by way of the blood vessels in the living kidney, has the property of staining the glomerular tufts intensely, and the stain can be easily fixed permanently by means of ammonium molybdate (Merck or Kahlbaum). According to Cowdry ('18), janus blue has the same property, but we have not employed this dye.

The animal is killed by bleeding from the carotid, the chest rapidly opened and a cannula inserted in the arch of the aorta. Through this 0.85 per cent salt solution is injected until the blood is well washed out of the kidney, when it is followed by a 1 in. 10,000 solution of janus green in salt solution. When the kidney is a uniform blue color, it is covered up by the intestines for fifteen to thirty minutes, to permit reduction of the excess dye. When this process is complete the kidney presents a purplish tint on its surface. Then a 5 per cent solution of ammonium molybdate is injected to check further reduction, and the kidney is removed and placed in a jar containing molybdate. In a successful preparation only the glomeruli will be deeply

stained blue, and as Cowdry (loc. cit.) remarked, they can be seen and counted even in thick pieces.

For counting it is necessary to separate the cortex from the medulla, except where a total count is to be made. This involves no great difficulty, since the cortex is deeply stained blue from the numerous glomeruli contained in it. To accomplish this, the kidney is cut into ten or twelve thick slices, out of which the medulla is carefully cut with a scalpel. When this is done a certain amount of fluid escapes, so it is important to determine at the same phase of the work the ratio of weight of sample counted to whole cortex. I have found also that pieces left in ammonium molybdate for some time change considerably in weight. As soon as the cortex is separated from the medulla the total weight is obtained, and a piece selected for counting and immediately weighed.

For counting, the method of teasing and compression is employed. The block to be counted is divided into small fragments which are teased apart and then compressed between two slides, or under a thick cover-glass. Counting is not difficult, and I am confident that the errors of counting are few. Doubtless some error is introduced by incomplete staining and by irregular oedema of the parts of the kidney. Another source of error is incomplete separation of medulla from cortex. As a check on the method of estimation, ten estimates and a complete count were made on the left kidney of a female rabbit weighing 1470 grams. The kidney contained by actual count 227,263 glomeruli. The average of the estimates was 212,269—an error of 6.7 per cent; but the error of the individual estimates varied from 19 per cent plus to 27.7 per cent minus. However, the error of six of the ten estimates was less than 9 per cent and of four less than 4 per cent. A plus error may arise through selecting for counting a piece from the surface of the cortex where the ratio of pars convoluta to pars radiata is high, and conversely a minus error may arise, as the table shows clearly, from incomplete separation of the medulla from cortex. By care in selection of the sample to be counted, the error may be reduced to less than 10 per cent. The results are contained in the subjoined tables.

The results of these counts and estimates are unexpectedly high. The actual content of glomeruli in a rabbit's kidney is tenfold that admitted for the kidney of the cat by Miller and Carlton, and more than one-third of the content found by Schweigger-Seidel in a pig's kidney fifteen times as heavy. Such differences, if true, suggest interesting physiological and anatomical implications, into the discussion of which, however, we cannot enter at present, pending the confirmation by newer and more accurate methods, of the results obtained by other investigators. This work is now in progress.

TABLE 1

NO.	SEX	BODY WEIGHT	KIDNEY WEIGHT[1]	CORTEX WEIGHT	WEIGHT OF SAMPLE	GLOMERULI	TOTAL
			gm.				
1	M	1990	Total count of one kidney				163075
2	M	1665	R 6.09	3.69	0.21	9221	161991
			L 6.56	3.73	0.19	8171	160390
3	F	2145	R 8.40	5.38	0.36	10460	156020
			L 8.68	5.54	0.42	11733	154566
4	M	2040	R 8.22	5.4	0.33	10326	168966
			L 8.27	5.6	0.175	3704	118160
5	M	2000	R 8.51	5.1	0.165	5405	166770
			L 8.24	4.6	0.290	10387	164680

[1] The weights of the kidney given in this table have no value except in relation to the counts, because different kidneys acquire different degrees of oedema in the process of staining.

TABLE 2

Female rabbit, weight 1470 grams

		Glomeruli
Total weight of kidney	9.98 grams	227,263
Total weight of cortex	4.85 grams	223,150
Total weight of medulla (plus cortex in separation)	5.13 grams	4,113

TABLE 2—*Continued*

Estimations

NUMBER	WEIGHT	MEDULLA PLUS OR MINUS	COUNT	ESTIMATE	PER CENT OF ERROR
1	0.160	Not observed	7,175	217,488	4.3 minus
2	0.140	Minus	7,843	270,846	19.1 plus
3	0.250	Plus	10,668	206,959	9.3 minus
4	0.280	Plus	9,482	164,228	27.7 minus
5	0.220	Minus	10,597	233,557	2.7 plus
6	0.250	Minus	11,868	230,439	1.3 plus
7	0.250	Minus	11,700	226,980	0.1 minus
8	0.290	Plus	12,395	207,244	8.8 minus
9	0.270	Plus	9,633	173,008	23.8 minus
10	0.230	Plus	9,081	191,427	15.7 minus
Average of the estimates				212,269	6.7 minus

LITERATURE CITED

BREMER, J. L. 1916 The interrelations of the mesonephros, kidney. and placenta in different classes of animals. Am. Jour. Anat., Phila., vol. 19, pp. 179–210.

BRODIE, T. G. 1914 A new conception of the glomerular function. Proceedings of the Royal Society, London, Ser. B, vol. 87, pp. 571-92.

COWDRY, E. V. 1918 The mitochondrial constituents of protoplasm. Contributions to Embryology, Carnegie Institution, Washington, vol. 8, pp. 40–148.

HUSCHKE, E. 1844 In Samuel Thomas von Sömmering's Lehre von den Eingeweiden und Sinnesorganen des menschlichen Körpers. Boss, Leipzig.

MILLER, W. S., AND CARLTON, E. P. 1894–95 The relation of the cortex of the cat's kidney to the volume of the kidney, and an estimation of the number of glomeruli. Transactions of the Wisconsin Academy of Sciences Arts and Letters, Madison, vol. 10, pp. 525-38.

PETER, K. 1907 Ueber die Nierenkanälchen des Menschen und einiger Säugetiere. Verhandlungen der Anatomischen Gesellschaft auf der einundzwangigsten Versammlung, Würzburg.

POLICARD, J. 1908–09 Le tube urinaire des Mammifères. Revue générale d'histologie, Paris, T. 3, pp. 307–568.

SCHWEIGGER-SEIDEL 1865 Die Nieren des Menschen und der Säugethiere in ihrem feineren Baue. Halle.